Original Article

Efficacy, Immunogenicity and Safety of a Human Rotavirus Vaccine RIX4414 in Singaporean Infants

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Abstract

Introduction: This was the first study conducted to evaluate the efficacy of 2 oral doses of the human rotavirus vaccine, RIX4414 in Singaporean infants during the first 3 years of life. Materials and Methods: Healthy infants, 11 to 17 weeks of age were enrolled in this randomised (1:1), double-blinded, placebo-controlled study to receive 2 oral doses of RIX4414 vaccine/placebo following a 0-, 1-month schedule. Vaccine efficacy against severe rotavirus (RV) gastroenteritis (Vesikari score ≥11) caused by wild-type RV strains from a period starting from 2 weeks post-Dose 2 until 2 and 3 years of age was calculated with 95% confidence interval (CI). Immunogenicity and safety of the vaccine were also assessed. Results: Of 6542 infants enrolled, 6466 were included in the efficacy analysis and a subset of 100 infants was included in the immunogenicity analysis. Fewer severe RV gastroenteritis episodes were reported in the RIX4414 group when compared to placebo at both 2 and 3 year follow-up periods. Vaccine efficacy against severe RV gastroenteritis at the respective time points were 93.8% (95% CI, 59.9 to 99.9) and 95.2% (95% CI, 70.5 to 99.9). One to 2 months post-Dose 2 of RIX4414, 97.5% (95% CI, 86.8 to 99.9) of infants seroconverted for anti-RV IgA antibodies. The number of serious adverse events recorded from Dose 1 until 3 years of age was similar in both groups. Conclusion: Two oral doses of RIX4414 vaccine was immunogenic and provided high level of protection against severe RV gastroenteritis in Singaporean children, during the first 3 years of life when the disease burden is highest.

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Key words: Diarrhoea, G and P types, Gastroenteritis, Intussusception

Introduction

Worldwide, rotavirus (RV) is the most common cause of severe dehydrating gastroenteritis (GE) among infants and young children aged 6 to 24 months.[1] RV disease causes significant morbidity and mortality;[2] in 2008, an estimated 453,000 children younger than 5 years of age died from RV diarrhoea, accounting for 37% of the 1.24 million diarrhoeal child deaths in this age group.[3] Further, 25% to 50% of diarrhoeal hospitalisations in both developed and developing nations and 23 million clinic visits in young children annually are due to RV diarrhoea.[4]

The consequences of infection due to RV vary depending on the healthcare facilities accessible to the population and the socioeconomic factors, which is apparent in Asian countries having diverse population and economies. According to the Asian Rotavirus Surveillance Network conducted across 9 Asian countries, RV was detected in 28% to 59% of hospitalised GE cases in children less than 5 years of age.[5] The high rate of hospitalisation due to RVGE has an enormous influence on healthcare costs and places a considerable burden on medical care services.[6]

Global surveillance studies have shown repeatedly that

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RV infects nearly all young children in both developed and developing countries.[7] Similar incidence rate of RV disease in diverse sanitary settings, despite the improvements in hygiene and water quality, further highlights the fact that vaccination is likely to be the most effective strategy that will aid in the reduction of RV disease burden worldwide.[5,8]

In an effort to reduce the global RV disease burden, an oral live attenuated human RV vaccine RIX4414 (Rotarix™, GlaxoSmithKline Vaccines, Belgium) was developed from the 89-12 candidate (cloned passage 43) containing the G1[P8] RV strain.[9-11] The lyophilised formulation of RIX4414 vaccine is currently licensed in over 100 countries. Phase III trials conducted across Europe and Latin America have demonstrated this vaccine to be well tolerated and efficacious in the first 2 years of life.[12,13]

This Phase III, multicentre study was conducted in high income countries of Asia, namely Singapore, Hong Kong and Taiwan to evaluate the efficacy, immunogenicity and safety of RIX4414 vaccine until 3 years of age. This paper presents the results of the Singapore cohort, while the overall study results of the Year 2 and 3 efficacy data are presented elsewhere.[14,15]

Materials and Methods

Study Design and Subjects

This multicentre, randomised, double-blind, placebo-controlled study was conducted in Singapore (NCT00329745). The study was conducted in accordance with Good Clinical Practice and adhered to all applicable local regulatory requirements including the Declaration of Helsinki. The study protocol and the consent forms were reviewed and approved by the ethics committee of the respective study centres and written informed consent was obtained from parents/guardians of all infants before any study procedures were initiated.

Healthy infants aged 11 to 17 weeks (at the time of Dose 1) were enrolled and randomised (1:1) into 2 treatment groups and subsequently received 2 oral doses of the RIX4414 vaccine or placebo following a 0-, 1-month schedule in Singapore. According to the current immunisation schedule in Singapore, 2 oral doses of RIX4414 are administered to children between 6 to 24 weeks of age with a minimum interval of 4 weeks between doses.[16] The routine vaccinations i.e. combined diphtheria-tetanus-acellular pertussis-inactivated poliovirus-*Haemophilus influenzae* type b (DTPa-IPV-Hib) vaccine was administered concomitantly with each RIX4414 vaccine/placebo dose. Administration of hepatitis B vaccine (HBV) and Bacille Calmette-Guérin (BCG) vaccine followed the national immunisation schedule of Singapore. Infants were excluded from the study if they had received any investigational drug/vaccine other than the study vaccine 30 days before Dose 1, had a history of allergy to any of the vaccine components or had immunosuppressive conditions. They were also excluded if they had a history of any clinically significant chronic gastrointestinal disease.

Study Vaccine

The RV vaccine, RIX4414 (Rotarix™), placebo and calcium carbonate buffer were manufactured by GlaxoSmithKline Vaccines, Rixensart, Belgium. One dose of RIX4414 vaccine contained not less than 106.0 median cell culture infective dose (CCID50) of live attenuated RIX4414 human RV strain. The composition of placebo was similar to that of the RIX4414 vaccine but without the vaccine strain. The lyophilised vaccine and placebo were reconstituted with the liquid calcium carbonate buffer prior to oral administration.

Assessment of Efficacy

The surveillance of GE episodes requiring overnight hospitalisation and/or rehydration therapy (equivalent to the World Health Organisation (WHO) Plan B or C) in a medical facility was carried out during the entire study period from Dose 1 until 3 years of age. Parents/guardians used diary cards to record GE episodes up to 2 days after the diarrhoea and vomiting had settled. If there were more than 5 symptom-free days between 2 occurrences of GE, parents/guardians were advised to consider these as 2 different episodes.

A GE episode was defined as occurrence of diarrhoea with or without vomiting. The severity of GE episodes was assessed using a 20-point Vesikari scale, where a score of ≥11 points was considered severe.[17] GE stool samples were collected preferably within 7 days of onset, during each GE episode. Collected stool samples were tested for the presence of RV using enzyme-linked immunosorbent assay (ELISA; Rotaclone™, Meridian Bioscience, USA) at GlaxoSmithKline Biologicals laboratories in Rixensart, Belgium. Furthermore, all RV-positive stool samples were tested by reverse transcriptase polymerase chain reaction (RT-PCR) followed by reverse hybridisation, to determine the G and P types at Delft Diagnostic Laboratory (DDL, The Netherlands).[18]

Assessment of Immunogenicity

The eligible infants were invited to participate in the immunogenicity subset and the first 100 infants (50 each in RIX4414 and placebo groups) with parental consent were part of the subset for immunogenicity. Blood was drawn at prevaccination and 1 to 2 months post-Dose 2, and tested

for anti-RV IgA antibody concentrations using an in-house ELISA test adapted from the assay developed by Prof RL Ward.[19] The assay cutoff was 20 units/millilitre (U/mL). A seropositive infant was defined as an infant whose IgA antibody concentration was ≥20 U/mL.

Assessment of Safety

Safety of the study vaccine was assessed in terms of serious adverse events (SAEs) including intussusception (IS), Kawasaki disease and fatal events starting from Dose 1 until 2 years of age. While safety was not an endpoint during the follow-up period between Year 2 and Year 3, investigators were asked to report any SAEs that were considered to be unusual or vaccine-related during this period.

Statistical Analyses

For the efficacy analysis (according-to-protocol [ATP] efficacy cohort), only wild-type RV (i.e. other than the vaccine strain) that were identified in an episode of GE requiring hospitalisation and/or rehydration therapy (equivalent to WHO Plan B or C) in a medical facility was considered. The ATP efficacy cohort included infants who had received 2 doses of RIX4414 vaccine/placebo, who had entered the efficacy follow-up periods and who had no RV other than the vaccine strain in their GE stool samples. The percentage reduction in the frequency of RVGE episodes in vaccinated infants when compared to the infants who received placebo was defined as vaccine efficacy (VE). VE was calculated using the formula (1 minus incidence of RVGE in the vaccine group/incidence of RVGE in the placebo group) with 95% confidence interval (CI). Efficacy analysis was performed from 2 weeks post-Dose 2 of RIX4414/placebo until 2 years of age (combined 2 year follow-up) and also from 2 weeks post-Dose 2 until 3 years of age (combined 3 year follow-up). VE against severe RVGE caused by circulating wild-type RV strains was calculated for the combined 2- and 3-year efficacy follow-up period.

For the immunogenicity analysis, the ATP immunogenicity cohort was considered. The ATP immunogenicity cohort comprised of infants who had complied with the protocol and for whom immunogenicity data were available at both prevaccination and 1 to 2 months post-Dose 2. One to 2 months post-Dose 2 of RIX4414 vaccine/placebo, the anti-RV IgA antibody seroconversion rate (anti-RV IgA antibody concentration ≥20 U/mL in infants previously seronegative) and geometric mean concentrations (GMCs) were tabulated with exact 95% CI.

Safety analysis was performed on total vaccinated cohort (TVC) which included all infants who had received at least 1 dose of RIX4414 vaccine/placebo. All statistical analyses were performed using SAS version 8.2 and 95% CI was calculated using Proc StatXact-5.

Results

Demographic Characteristics

A total of 6542 infants were enrolled between December 2003 and August 2005 to receive 2 doses of either the RIX4414 vaccine (n = 3274) or the placebo (n = 3268). The ATP cohort for efficacy included 6466 infants (3237 infants in the RIX4414 and 3229 infants in the placebo group) at 2 and 3 years of age (Fig. 1). Of these, a subset of 100 infants was included in the immunogenicity analysis.

The mean age of vaccinated infants at the time of Dose

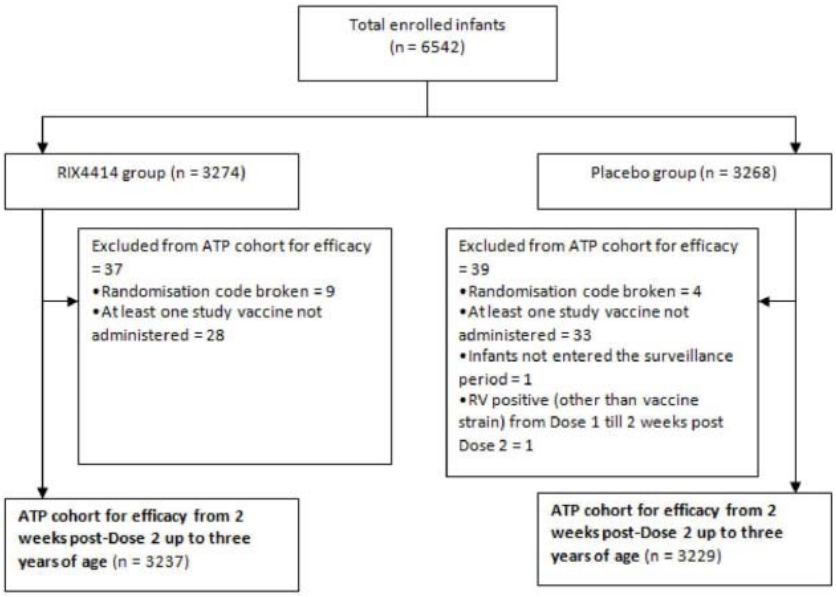

Fig. 1. Consort flowchart.
ATP: According-to-protocol; RV: Rotavirus.

1 of vaccine/placebo was 13.3 ± 0.86 weeks and 18.3 ± 1.29 weeks at Dose 2 of RIX4414 vaccine/placebo (TVC). The main race categories were Chinese (62.5%), Malay (27.9%) and Indian (7.6%). The ratio of females (50.3%) and males (49.7%) was similar in both groups. For the majority (>99%) of infants, DTPa-IPV-Hib vaccine was co-administered with both the doses of RIX4414 vaccine/placebo (Table 1).

Table 1. Co-administered Vaccines

Co-administered Vaccination at Dose 1 of RIX4414 Vaccine/Placebo			
Categories	**RIX4414 n* = 3274 n (%)†**	**Placebo n* = 3268 n (%)†**	**Total n* = 6542 n (%)†**
Any	3270 (99.9)	3261 (99.8)	6531 (99.8)
BCG	0 (0.0)	1 (0.0)	1 (0.0)
DTPa + HBV + HIB + IPV	1 (0.0)	1 (0.0)	2 (0.0)
DTPa + IPV + HIB	3268 (99.8)	3259 (99.7)	6527 (99.8)
HBV	13 (0.4)	10 (0.3)	23 (0.4)
Co-administered Vaccination at Dose 2 of RIX4414 Vaccine/Placebo			
Categories	**RIX4414 n = 3246* n (%)†**	**Placebo n = 3235* n (%)†**	**Total n = 6481* n (%)†**
Any	3246 (100)	3229 (99.8)	6475 (99.9)
DTPa + HBV	1 (0.0)	0 (0.0)	1 (0.0)
DTPa + HBV + HIB + IPV	1 (0.0)	0 (0.0)	1 (0.0)
DTPa + IPV + HIB	3244 (99.9)	3229 (99.8)	6473 (99.9)
HBV	1 (0.0)	1 (0.0)	2 (0.0)

*Total number of infants having received the considered dose of RIX4414 vaccine/placebo.

†Number/percentage of infants who received the specified vaccination on the same day as the considered dose of RIX4414 vaccine/placebo.

Vaccine Efficacy

From 2 weeks post-Dose 2 until 2 years of age, VE against severe RVGE was 93.8% (95% CI, 59.9 to 99.9) in the RIX4414 group (Table 2). Similarly, during the follow-up period from 2 weeks post-Dose 2 until 3 years of age, VE against severe RVGE was 95.2% (95% CI, 70.5 to 99.9) in the RIX4414 group (Table 2).

The RV types isolated from severe RVGE episodes during the period from 2 weeks post-Dose 2 until 3 years of age were G1P[8], G2P[4], G3P[8] and G9P[8] in the placebo group vs G9P[8] in the RIX4414 group. There was no presence of vaccine virus and wild-type G1P[8] isolated from the severe RVGE stool samples in the RIX4414 group (Table 3).

Immunogenicity

All initially seropositive infants and infants with unknown seropositivity status at prevaccination were eliminated from both groups for the purpose of ATP immunogenicity analysis. Therefore, all infants included in the ATP immunogenicity cohort were seronegative for RV at prevaccination in the RIX4414 and placebo groups. The percentage of infants with anti-RV IgA antibody concentration ≥20 U/mL 1 to 2 months post-Dose 2 was 97.5% (95% CI, 86.8 to 99.9) in the RIX4414 (n = 40) and 2.2% (95% CI, 0.1 to 11.5) in the placebo groups (n = 46). The observed GMCs in all infants 1 to 2 months post-Dose 2 were 368.5 U/mL (95% CI, 231.0 to 588.0) and <20 U/mL in the RIX4414 (n = 40) and placebo groups (n = 46), respectively. The serum anti-RV IgA GMCs calculated on seropositive infants were 404.3 U/mL in the RIX4414 group (n = 39) and 119.0 U/mL in the placebo group (n = 1).

Safety

During the combined 2-year follow-up period, at least 1 SAE was recorded in 429 infants in the RIX4414 group and 466 infants in the placebo group. The most frequently

Table 2. Vaccine Efficacy against Severe Rotavirus Gastroenteritis Caused by Circulating Wild-type Rotavirus Strains From 2 Weeks Post-Dose 2 Up To 2 Years and 3 Years of Age (ATP Cohort for Efficacy)

Follow-up	**Group**	**n***	**n**	**%† (95% CI§)**	**Vaccine Efficacy % (95% CI§)**	***P* Value‡**
Year 2	RIX4414	3237	1	0 (0 – 0.2)	93.8 (59.9 – 99.9)	<0.001
	Placebo	3229	16	0.5 (0.3 – 0.8)	-	-
Year 3	RIX4414	3237	1	0 (0 – 0.2)	95.2 (70.5 – 99.9)	<0.001
	Placebo	3229	21	0.7 (0.4 – 1)	-	-

*Number of infants included in each group.

†Number/percentage of infants reporting at least one severe rotavirus gastroenteritis episode.

‡Two-sided Fisher's exact test (significant level of $\alpha = 0.05$).

§Exact 95% confidence interval.

Table 3. Number of Severe Rotavirus Gastroenteritis Episodes Reported during the 3-year Efficacy Follow-up by G and P Types (ATP Cohort for Efficacy)

Type	RIX4414 n = 1*		Placebo n = 21*	
	n	%†	n	%†
Any	1	100	21	100
G1WT + P8WT	0	0.00	10	47.62
G2 + P4	0	0.00	2	9.52
G3 + P8WT	0	0.00	5	23.81
G9	0	0.00	1	4.76
G9 + P8WT	1	100	3	14.29

*Number of severe rotavirus gastroenteritis episodes.
†Number/percentage of severe rotavirus gastroenteritis episodes, by type.
WT: Wild-type

recorded SAEs were bronchiolitis (96 infants in the RIX4414 group and 119 infants in the placebo group) followed by GE (37 infants in the RIX4414 group and 50 infants in the placebo group). Four vaccine-related SAEs were reported—1 SAE (GE; placebo group) reported post-Dose 1, while 3 SAEs (RVGE [placebo group], urticaria [RIX4414 group] and viral GE [RIX4414 group]) were reported post-Dose 2.

There were reports of 3 fatal SAEs (all in placebo group): aspiration and metabolic disorder (1), adenoviral pneumonia (1) and interstitial lung disease (1). None of these fatal SAEs was assessed as causally related to vaccination by the investigator.

Definite IS cases were reported after Dose 2 in 3 male infants (1 in the RIX4414 group and 2 in the placebo group) during the combined 2-year follow-up period and 1 IS case (RIX4414 group) was reported between Year 2 and Year 3. All IS cases resolved and were assessed as not related to vaccination. The IS cases were reported during the 31-day follow-up period after each vaccine/placebo dose.

Kawasaki disease was recorded in 8 infants in the RIX4414 and 4 infants in the placebo groups during the combined 2-year follow-up period; all these cases occurred after the second vaccine/placebo doses. However, no Kawasaki disease was reported during the time period between Year 2 and Year 3.

Discussion

This was the first study in Singapore where the efficacy of the RV vaccine against severe RVGE was demonstrated during the first 3 years of life of children, when the disease burden is at its highest.[1] The present study showed that in the Singaporean setting, 2 doses of the live attenuated RV vaccine (RIX4414) afforded high level of protection against severe RVGE until 2 (VE = 93.8%) and 3 years of age (VE = 95.2%). These results were comparable to VE results observed in a 2-year efficacy study conducted in Europe (VE = 90.4%) but higher than that observed in Latin America (VE = 80.5%).[12,13] These efficacy results are also in line with the overall Asian multicountry results.[14,15]

RV remains the viral agent primarily responsible for acute GE among children less than 5 years of age in Singapore. In this study, a protective efficacy of 93.8% against severe RVGE in the first 2 years of life suggested that RV vaccination programmes are important and they may have potential public health impact in reducing the RV disease burden. Although the efficacy demonstrated by RIX4414 vaccine is clearly suggestive of the high level of protection yielded by the vaccine, these results may not be generalised to the whole Asian population since Asia comprises not only of high income countries like Singapore but also several impoverished low income countries. In addition to high VE of RIX4414 vaccine, the anti-RV IgA seroconversion rate observed was also high (97.5%) 1 to 2 months post-Dose 2 which were similar to results obtained in an earlier Singaporean study.[6,20]

Safety data have revealed that there was no evidence for a clinically significant difference between the RIX4414 vaccine group and the placebo group for SAEs reported from Dose 1 until 3 years of age. There were 4 cases of IS reported during this study. Epidemiological studies in Singapore over an 8-year period has indicated an IS incidence rate of 60 per 100,000 and 32 per 100,000 in <1-year-old children and <2-year-old children, respectively.[21] Furthermore, evidence from a large safety study conducted in Latin America and Finland with 63,225 infants, indicated that the RIX4414 vaccine was not associated with an increased risk of IS.[22] Keeping the background IS rate in perspective, the RV vaccine used in this study was not associated with an increased risk of IS compared to the placebo in Singaporean infants.

Due to the potential association of RV vaccination and Kawasaki disease, the Global Advisory Committee on Vaccine Safety recommended the monitoring of Kawasaki disease in all the RV vaccine trials. Although there have been no reports of Kawasaki disease in the European Union after the administration of 12 million doses of the RIX4414 vaccine, Kawasaki disease is still monitored in all ongoing trials.[23] In this study, the incidence of Kawasaki disease reported was similar in both the RIX4414 and placebo groups, with no causal association to vaccination.

A limitation of our study was that it was only powered to observe a significant difference in the incidence of severe RVGE for the pooled countries included in the study and not for individual countries. Moreover, it is possible that a

small proportion of RV infections may have gone undetected given that the sensitivity of the assay used in this study was recently reported to have 76.8% sensitivity compared to 100% sensitivity reported by the manufacturer.[24]

While improvements in sanitary conditions and hygienic practises have indeed rendered extraordinary public health benefits in Asian countries, these steps seem to have very little effect on the incidence of RV disease. Effective immunisation early in life of infants that can offer sustained protection against severe RVGE may aid in curbing the disease burden in this region.

Conclusion

This study conducted in Singapore demonstrated that the 2 oral doses of RIX4414 vaccine was immunogenic, safe and provided high protection against severe RVGE during the first 3 years of life. Given its high efficacy, this RV vaccine can be expected to have a considerable public health impact in high income Asian countries by reducing the RV disease burden and in turn hospitalisation due to GE.

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Conflict of Interest

The authors declare the following conflict of interest: PV Suryakiran and Htay Htay Han are employees of GlaxoSmithKline (GSK) group of companies. Hans L Bock and Yee Leong Teoh were employees of GlaxoSmithKline Group of companies at the time of protocol conception, study conduct and manuscript initiation and held shares of GSK. Yee Leong Teoh was employed at the National Healthcare Group Polyclinics, and was an investigator on this study. However, at the time of the ending of the studies and during manuscript preparation, he was employed by the GlaxoSmithKline group of companies. The other authors have no conflict of interest to declare.

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